Vasoactive Intestinal Peptide Stimulates Prolactin mRNA Expression in Turkey Pituitary Cells: Effects of Dopaminergic Drugs (43991)

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Abstract. **It is well documented that vasoactive intestinal peptide (VIP) is a prolactin (PRL)–releasing factor and that dopamine (DA) is an inhibitory neurotransmitter in avian species. However, the roles of VIP and DA in the regulation of PRL gene expression are unclear. In this study, primary anterior pituitary cells cultured from laying turkeys were utilized to investigate the influence of VIP and dopaminergic D_1 and D_2 receptors on PRL secretion, PRL mRNA, and PRL synthesis. Incubation of pituitary cells with VIP increased PRL secretion up to 3.5-fold within 3 hr. Prolactin mRNA was undetectable during the first 2 hr of pituitary cell treatment; thereafter, the PRL mRNA content response to VIP increased within 24–48 h ($P < 0.05$). Total PRL content (media + cellular) increased over time in the presence of VIP. The response of cells incubated in the presence of a dopaminergic D_1 receptor agonist (SKF38393) was variable and inconclusive. However, cells incubated with a dopaminergic D_2 receptor agonist (quinpirole) inhibited VIP-induced PRL secretion ($P < 0.05$) and PRL mRNA levels ($P < 0.05$) in a dose-related fashion without effect on the basal levels of PRL release and PRL mRNA. These observations suggest that VIP, in addition to acting as a PRL-releasing peptide, also plays a role in the regulation of PRL gene expression. Moreover, the results of this study also indicate that a drug that can selectively stimulate dopamine D_2 receptors can also regulate PRL secretion and PRL mRNA in turkey pituitary cells in culture.** [P.S.E.B.M. 1996, Vol 212]

Vasoactive intestinal peptide (VIP) is a neuropeptide that was initially isolated from porcine duodenum (1). In mammals, studies have shown that VIP is involved in the regulation of prolactin (PRL) secretion from the pituitary gland (2, 3). Vasoactive intestinal peptide stimulates PRL release *in vivo* (4, 5) and *in vitro* (6–8). The administration of VIP antiserum inhibits PRL release induced by stress, serotonin, or suckling (9–12), suggesting that VIP is a PRL-releasing factor.

In avian species, VIP is the only substance found to be a consistent and potent PRL releaser (13–18). The levels of plasma PRL and pituitary PRL mRNA, as well as VIP immunoreactivity in the hypothalamus, are correlated during the turkey reproductive cycle (19, 20). Vasoactive intestinal peptide neurons in the hypothalamus increase under conditions of hyperprolactinemia. Passive immunization with VIP antiserum depresses plasma PRL and terminates incubation behavior in incubating chickens (21, 22). Active immunization of female turkeys with VIP prevents the secretion of PRL induced by electrical stimulation of the hypothalamus (17) and prevents incubation behavior

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and its associated rise in plasma PRL levels (23). In addition to acting as a PRL-releasing hormone, VIP also appears to regulate PRL mRNA levels in chickens and turkeys (22, 23).

In contrast to this central stimulatory control in birds, it is well established that PRL secretion in mammals is under tonic inhibitory control by dopamine (DA) (24, 25). There are several lines of evidence showing that DA agonists also have a negative influence on the regulation of PRL gene transcription. The DA agonist bromocryptine suppresses PRL release and the steady-state levels of PRL mRNA in the rat pituitary (26, 27), and attenuates PRL mRNA polyadenylation (28), whereas DA antagonists increase the level of PRL mRNA (29). Furthermore, dopamine agonists are able to induce parallel inhibition of PRL synthesis and transcription of the PRL gene in primary cultures of female rat pituitary cells (30–32). In addition to its inhibitory role, DA is also capable of stimulating PRL release from lactotrophs at low concentrations *in vivo* (33) and *in vitro* (34–37).

Reproductive activity in birds is readily influenced by pharmacological manipulation of the central monaminergic nervous system (38). Catecholamines have an inhibitory influence on PRL release in the turkey (39). Dopamine inhibits PRL release from chicken, pigeon, and turkey anterior pituitary cells *in vitro* (40–43). Electrical stimulation–induced PRL release is diminished by the DA agonist apomorphine and is potentiated by the DA antagonist pimozide (44). However, the administration of DA agonists increases plasma PRL in cockerels and young turkeys (40).

The regulation of PRL gene expression in avian species by DA remains largely unexplored, and evidence for VIP to play a role in such regulation is limited. This study was therefore conducted to clarify the involvement of VIP and DA in the regulation of PRL gene expression in the turkey by using a cDNA encoding turkey PRL (20) as a hybridization probe to measure steady-state levels of PRL mRNA in cultured primary turkey pituitary cells.

Materials and Methods

Animals, Tissue Collection, and Culture Conditions. Laying female turkeys (Nicholas Large White strain) were used in these experiments. The birds were kept on a 15:9-hr light:dark photoregimen in a temperature controlled (15°–21°C) group housing unit with constant access to feed and water. Pituitary cells were obtained using a modified trypsin/neuraminidase procedure (45, 46). Briefly, anterior pituitaries were isolated, minced, and placed in a Krebs-Ringer bicarbonate (KRB) buffer solution supplemented with amino acids (Eagle's minimum essential amino acids; Difco, Walkersville, MD), 0.3 mg/ml L-glutamine (Sigma Chemical Co., St. Louis, MO), 2.5 mg/ml α-D($^+$) glucose (grade III, Sigma), 3 mg/ml bovine serum albumin (BSA, fraction V; Sigma), and 0.1 mg/ml gentamycin sulfate (Sigma). The pituitary fragments were enzymatically digested with 1 mg/ml trypsin (bovine pancreas type III; Sigma) and 2 μg/ml deoxyribonuclease I (DNase I, type I; Sigma) for 20 min at 37°C in a shaking water bath. The supernatant was removed and replaced by the supplemented KRB solution containing 1 mg/ml trypsin inhibitor (soybean isolate, type II-s; Sigma). The pituitary fragments were incubated for 5 min at 37°C and centrifuged. The supernatant was removed and replaced by a Ca^{++}- and Mg^{++}-free supplemented KRB solution containing 0.7 mg/ml Na_2 EDTA. After a 15-min incubation at 37°C, the pituitary fragments were mechanically dispersed and the resulting cell suspension was filtered through a 60-μm mesh gauze to remove undigested tissue. Following centrifugation (10 min at 50*g*), the supernatant was decanted and the cell pellet was resuspended in tissue culture medium consisting of M-199, 0.35 mg/ml sodium bicarbonate, 4.8 mg/ml HEPES, 1 μg/ml insulin (Sigma), 3% fetal bovine serum, 3% turkey poult serum, 0.05 mg/ml gentamycin sulfate, 100 U/ml penicillin, and 0.1 mg/ml streptomycin, pH 7.4. Cell viability was approximately 95% using the trypan blue dye exclusion procedure following a 24-hr incubation at 40°C. Cells were aliquoted to contain the desired number of cells in 1 ml and incubated in suspension in glass culture tubes in a Dubnoff Shaker-Bath Incubator. The cultures were exposed to 95% O_2/5% CO_2 as necessary to maintain the pH at 7.4.

Radioimmunoassay, RNA Analysis, and Northern Hybridization. At the conclusion of incubation, cells were separated by centrifugation and the supernatant retained and stored at −20°C until PRL was assayed by homologous radioimmunoassay (47). The cell pellet was resuspended in 1 ml of 0.1% BSA–phosphate-buffered saline (PBS) and 100 μl was similarly stored for PRL assay. The remaining cell suspension was pelleted and the cells were washed with Ca^{++}-, Mg^{++}-, and RNase-free PBS and stored in autoclaved microcentrifuge tubes at −80°C for RNA extraction. Total RNA was isolated (48), suspended in DEPC-treated sterile water, and quantified by ultraviolet spectrometry. Triplicate RNA samples (1 μg/slot) from each treatment were denatured in formamide and immobilized on nitrocellulose paper using a slot-blot manifold (Scheicher & Schuell, Inc., Keene, NH). Ten 1:1 serial dilutions of 500 ng of a turkey PRL cDNA insert (20) were denatured and blotted as above for use as a standard curve. Filters were baked for 2 hr at 80°C, then prehybridized for 6 hr in formamide solution (50% deionized formamide, 2 × SSC [0.3 *M* NaCl, 0.03 *M* sodium citrate, pH 7], 0.05 *M* sodium phosphate (pH 6.5), 0.02% polyvinylpyrrolidone (mol wt: 40,000), 0.02% BSA, 0.02% ficoll (mol wt: 400,000),

>100 μg/ml denatured transfer RNA). Filters were then hybridized overnight at 42°C in a fresh formamide solution with constant agitation with a nick-translated ^{32}P-labelled turkey PRL cDNA (average specific activity: 3×10^8 dpm/μg). Following hybridization, filters were washed twice in 2 × SSC at 25°C for 5 min and then twice in 2 × SSC-1% SDS at 65°C for 30 min. The final wash was with 0.2 × SSC-0.1% SDS for 30 min. All washes employed constant agitation. After hybridization and washing, the filters were air dried and counted using an Ambis 4000 Radioanalytic Image Detector (Ambis, Inc., San Diego, CA). The amount of RNA hybridized to the PRL probe from each treatment was determined by comparison to the PRL cDNA standard curve. The amount of PRL mRNA determined from the standard curve was normalized to the amount of total cellular RNA applied to the slot blot on a per microgram basis. Values reported for each sample were an average of the triplicate samples that were slot-blotted.

We also selected one RNA sample from each treatment for Northern analysis to verify our slot-blot results and to monitor the intactness of the RNA. RNA samples were mixed with loading buffer (50% deionized formamide, 16% formaldehyde, 1 × MOPS, 6% glycerol, 10% bromphenol blue/xylene cyanole) and were loaded on a 1% agarose-formaldehyde gel prepared according (49). Cellular chicken RNA with ethidium bromide was loaded as a marker. Following electrophoresis (60 V for 6–8 hr), the gel was washed twice (15 min) in 0.025 *M* sodium phosphate buffer, pH 6.5, with a constant agitation at room temperature. Each gel was photographed and the RNA was transferred onto GeneScreen (Dupont, Boston, MA) using the capillary blot procedure described by Dupont. All samples from a single experiment were fractionated by electrophoresis on the same gel. The blots were baked for 2 hr at 80°C under vacuum. Blots were prehybridized, hybridized with labeled PRL cDNA probe, and washed as described above and prepared by autoradiography. Blots were exposed to Kodak XAR 5 film (X-Omat AR; Eastman Kodak Co., New Haven, CT) for 6–24 hr at −80°C using cassettes equipped with intensifying screens (Dupont). After hybridization with turkey PRL cDNA probe, the blots were stripped and hybridized to the poly(U) probe as described by Shi *et al.* (50). The PRL cDNA-hybridized and poly(U)-hybridized blots were counted with the Ambis Analyzer and the data from the poly(U) scans were used to normalize the amount of RNA in each sample. Based on this normalization, the scans of the PRL cDNA-hybridized blots were used to quantify the abundance of PRL mRNA in each sample.

VIP Effects on PRL and PRL mRNA. Three experiments were conducted to refine the culture parameters and evaluate the effects of VIP on PRL secretion and content, and on cellular PRL mRNA levels. The effects of cell density and VIP concentration were examined by incubating cells (0.25, 0.5, 1, or 2×10^6 cells) with varying concentrations of porcine VIP (0, 10^{-12}, 10^{-10}, 10^{-8}, 10^{-6} *M*). The media used was M-199 containing 0.1% BSA, and incubations were concluded after 3 hr. In a second experiment, the effect of time in culture prior to treatment was assessed by incubating cells for 12, 24, 48, or 72 hr after transfer to culture tubes and then stimulating with 10^{-6} *M* VIP for 3 hr. In this experiment, the initial incubation was in M-199 containing 3% fetal bovine serum, 3% charcoal-stripped turkey poult serum, 1 μg/ml insulin, and 5 μg/ml Amphotericin B. VIP stimulation was accomplished in media consisting of M-199 with 0.1% BSA as before. In a third experiment, the effect of duration of stimulation by VIP was studied by incubating cells with (10^{-6} *M*) or without porcine VIP for 3, 6, 12, 24, 48, or 72 hr. The medium used was the serum-containing medium described above.

Blockade of the PRL Response to VIP. The specificity of the VIP effect on PRL and PRL mRNA was studied by preincubating the cells with a VIP receptor antagonist (4-Cl-D-Phe$_6$,Leu$_7$-VIP) (51) prior to VIP stimulation. Cells were incubated for 48 hr in serum-containing medium, pelleted, and resuspended in M-199-0.1% BSA with or without 10^{-5} *M* VIP receptor antagonist (ATG). After a 20-min preincubation period, the medium was again replaced and the cells were incubated for 3 hr in the presence or absence of 10^{-9} *M* chicken VIP. In the same experiment, additional treatments tested the ability of immunoneutralization of VIP. VIP-containing media was incubated for 1 hr with either normal sheep serum (NSS) or VIP antiserum (AS) (21) prior to addition to the pituitary cells. The ability of the preincubated media to stimulate PRL or PRL mRNA was then determined.

Effects of DA Receptor Agonists. We studied the effects of the D$_1$ DA receptor agonist, SKF38393, and the D$_2$ DA receptor agonist, quinpirole, on VIP-mediated changes in PRL and PRL mRNA. First, in separate experiments, the dose-response effects of the agonists were studied in cells incubated for 48 hr in serum-containing medium and then resuspended in test medium (M-199-0.1% BSA) containing receptor agonist. After 15 min preincubation with agonist, chicken VIP was added to a final concentration of 0 or 10^{-9} *M* and incubation was continued for 3 hr. The DA receptor agonists were tested at final concentrations of 0, 10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, or 10^{-4} *M*. Prolactin was measured in media and cells, and PRL gene expression was assessed by Northern analysis. Based on the results of these experiments, a final experiment was designed to confirm the effects of 10^{-6} quinpirole on PRL secretion and to quantify its effects on PRL mRNA abundance.

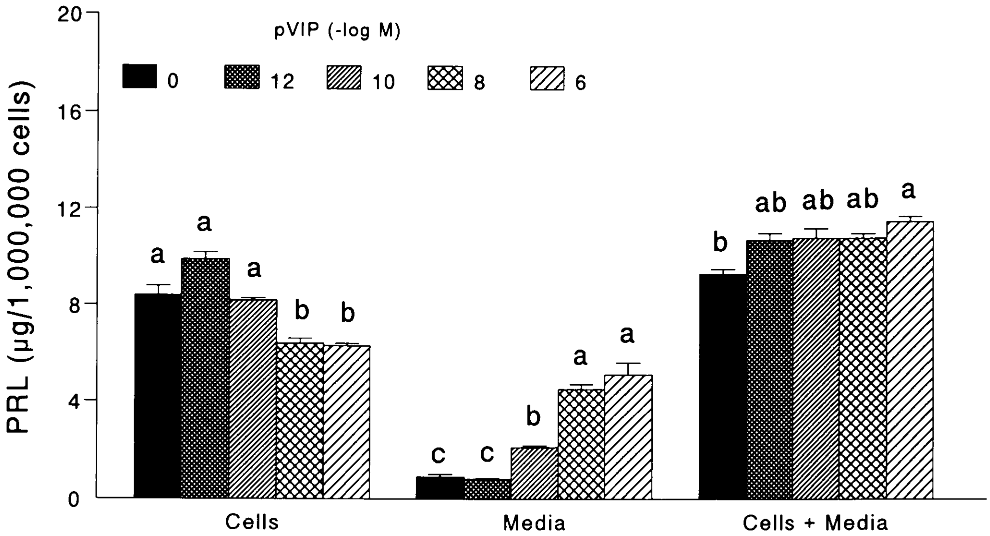


Figure 1. Effect of porcine VIP concentrations on PRL secretion, PRL content, and total PRL of turkey pituitary cells cultured *in vitro* for 3 hr. Means + SEM are shown. Comparisons were made among VIP concentrations. Columns with different letters are significantly different ($P < 0.05$).

Statistical Analysis. Each experiment was conducted twice ($n = 2$), and there were four replicates per treatment within each experiment. The data from radioimmunoassay and slot-blot were analyzed using the General Linear Model procedure in the Statistical Analysis System (52). Significant differences in mean PRL or PRL mRNA levels between treatment groups were separated using Duncan's Multiple Range test at a significance level of $\alpha = 0.05$.

Results

For the sake of brevity, Figure 1 shows the results of the one million cell treatment only. The amount of PRL secreted by cultured turkey pituitary cells increased with increasing cell numbers (data not shown) and with increasing VIP concentrations. Of the doses tested, 10^{-10} *M* was the lowest dose of VIP to stimulate PRL secretion above control levels, while 10^{-8} and 10^{-6} *M* porcine VIP produced a maximum PRL response. Cellular PRL was significantly reduced at each of the above doses of VIP. Total PRL (media + cells) increased in response to the highest dose of VIP. Northern blot analysis revealed low levels of PRL mRNA in cultures of 2×10^6 cells at all levels of VIP treatment, but PRL mRNA was undetectable by Northern blot analysis in cultures containing fewer cells (data not shown).

Preincubation of cells for 12, 24, 48, or 72 hr prior to VIP stimulation had no significant effect on the cell's subsequent response to a 3-hr VIP challenge (data not shown). At any time point, 10^{-6} *M* porcine VIP significantly stimulated PRL secretion and depleted cellular PRL content without altering total PRL. However, Northern blot analysis demonstrated a clear stimulatory effect of VIP on PRL mRNA levels if the cells were first preincubated for 48 or 72 hr prior to the VIP challenge (Fig. 2). Interestingly, PRL mRNA was not present at a detectable level in either control or VIP-stimulated cultures which had been preincubated for less than 48 hr.

Extended exposure of cultured pituitary cells to VIP resulted in a progressive increase in PRL secretion into the media for 48 hr, accompanied by a depletion of cellular PRL stores (Fig. 3, a and b). However, cellular PRL levels stabilized by 12 hr, and increased slightly between 48 and 72 hr. Concomitantly, PRL mRNA levels were significantly higher in VIP-treated cells than in control cells after 6 hr of incubation with VIP ($P < 0.05$). Prolactin mRNA levels in VIP-treated cells increased markedly between 12 and 24 hr of VIP treatment, and remained at a high level at 48 hr (Fig.

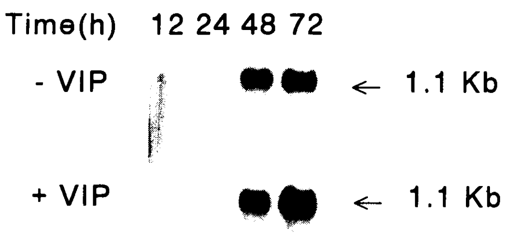
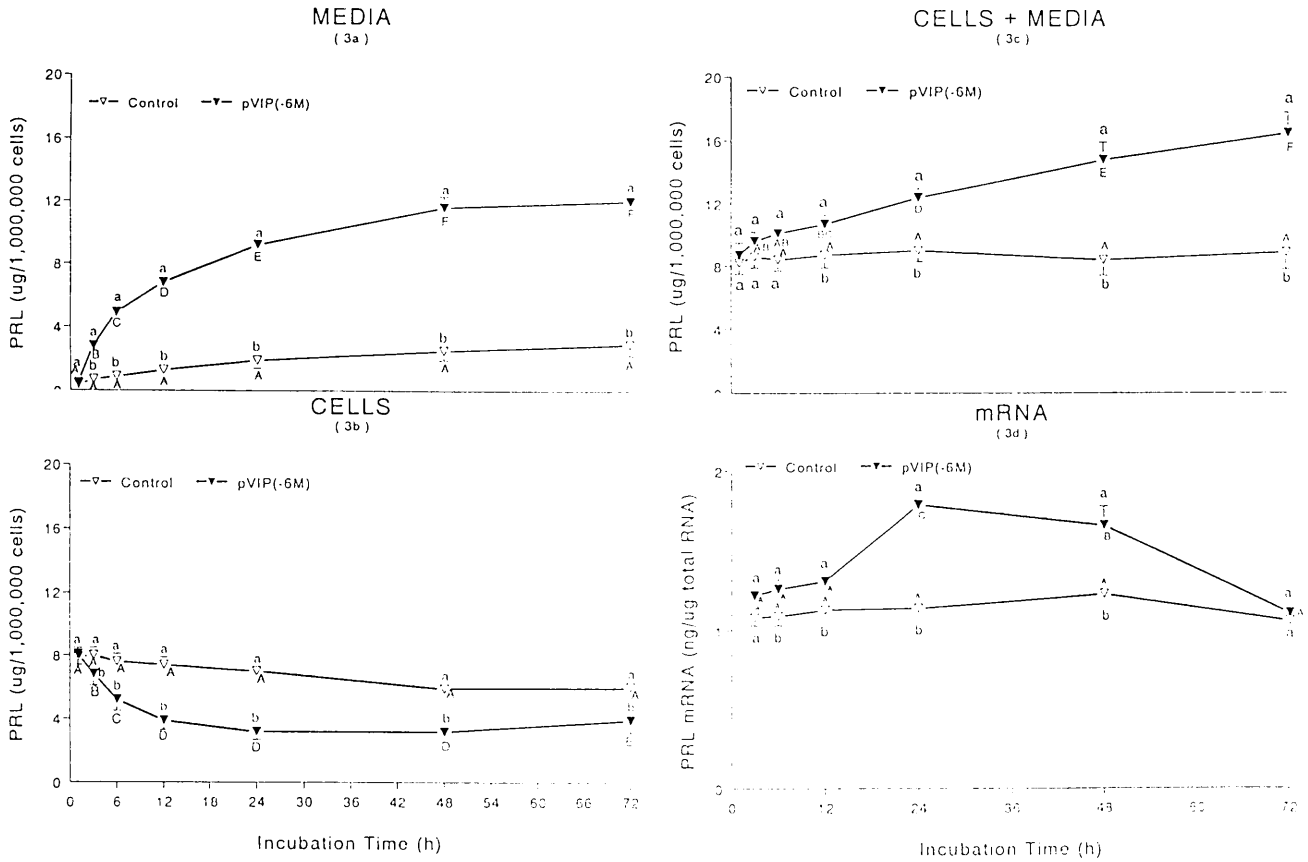


Figure 2. Effect of preincubation time (12, 24, 48, 72 hr) prior to 3-hr stimulation of cultured turkey pituitary cells with 0 or 10^{-6} *M* VIP on the amount of PRL mRNA. Northern hybridization analysis of cellular PRL mRNA is shown. Each lane represents total RNA from 90% of the cells cultured.



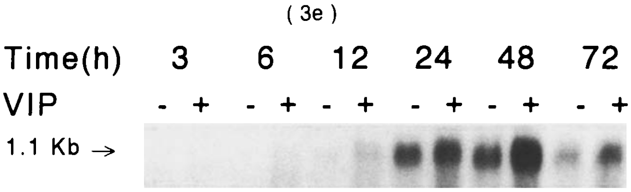


Figure 3. Effect of incubation time on PRL secretion (a), PRL cell content (b), total PRL (c), and PRL mRNA levels (d) from pituitary cells cultured in the presence or absence of 10^{-6} *M* porcine VIP. Means + SEM are shown. Comparisons made between times within each treatment are identified by capital letters while differences between treatments are identified by lowercase letters. Different letters indicate a significant difference ($P < 0.05$). There were four replicate tubes/treatment and $n = 2$ experiments, with the exception of PRL mRNA where three replicates were used. Northern hybridization analysis of cellular PRL mRNA is shown in Panel e. Each lane represents total RNA from 90% of the cells cultured.

3d). Total PRL content was elevated relative to nonstimulated controls after 12 hr of VIP treatment, and increased significantly at each subsequent time point (Fig. 3c). Neither PRL nor PRL mRNA levels changed significantly during 72 hr of incubation in unstimulated cultures. Northern blot analysis was unable to detect PRL mRNA during the first 12 hr of incubation, but a clear stimulation of PRL mRNA by VIP was observed after 24, 48, and 72 hr of incubation (Fig. 3e). Even though slot-blot analysis indicated a lack of significant change in PRL mRNA during 72 hr of incubation of control culture (Fig. 3d), there was an indication of an increased bands' intensity with advance incubation time as detected by Northern blot analysis (Fig. 3e).

Incubation of pituitary cells with 10^{-9} *M* chicken VIP induced 3.5-fold greater PRL secretion than that observed in untreated cultures ($P < 0.0001$; Fig. 4a). This PRL response to VIP was not altered by prior incubation of the VIP with normal sheep serum, but similar incubation with an antiserum to VIP completely blocked VIP-stimulated PRL secretion. Preincubation of the pituitary cells with a VIP receptor antagonist depressed the PRL response to VIP stimulation ($P < 0.0001$; Fig. 4a). No significant treatment differences in cellular or total PRL content (medium + cells) were observed (data not shown). Prolactin mRNA levels increased significantly in response to chicken VIP ($P < 0.0006$; Fig. 4b). Preincubation of VIP with normal sheep serum had a slight but nonsignificant effect on the ability of VIP to stimulate PRL mRNA levels, while VIP antiserum markedly reduced the PRL mRNA response to VIP ($P < 0.001$). Similarly, the VIP-stimulated mRNA levels were also depressed ($P < .001$) by prior treatment of the pituitary cells with VIP receptor antagonist (Fig. 4b). Northern blot analysis (Fig. 4c) indicated that VIP antiserum was more effective than VIP receptor antagonist in reducing the PRL mRNA response to VIP, and, further, that VIP antiserum also reduced PRL mRNA levels in control (unstimulated) cells.

The D_1 receptor agonist, SKF38393, had a variable effect on VIP-stimulated PRL secretion (Fig. 5a). The increase in PRL secretion due to VIP was slightly depressed by 10^{-10} *M* SKF38393, but slightly increased by 10^{-4} *M* SKF38393. Decreased cellular and total PRL were observed at low doses of agonist ($P < 0.05$; Fig. 5, b and c). Northern analysis (Fig. 5d) revealed no clear effect of D_1 receptor agonist on VIP-stimulated PRL mRNA levels.

The D_2 receptor agonist, quinpirole, consistently depressed ($P < 0.0002$) VIP-stimulated PRL secretion at high doses (10^{-8} *M* to 10^{-4} *M;* Fig. 6a). Basal PRL secretion was significantly depressed by 10^{-6} and 10^{-4} *M* quinpirole. However, this agonist showed a slight stimulatory effect on basal and VIP-induced PRL secretion at 10^{-10} *M* (Fig. 6a; $P < 0.002$). The effect of quinpirole on cellular and total PRL varied with dose (Fig. 6, b and c). Northern analysis also suggested an inhibitory effect of DA D_2 receptor agonist on VIP-stimulated PRL mRNA levels (Fig. 6d). The suppressive effects of quinpirole on VIP-stimulated PRL secretion and (particularly) mRNA levels was confirmed by a final experiment focusing on the effects of 10^{-6} *M* quinpirole (Fig. 7). In this experiment, the D_2 receptor agonist slightly suppressed VIP-stimulated PRL secretion ($P < 0.001$; Fig. 7a) without significantly affecting basal PRL secretion or total PRL (Fig. 7c). A reduction in cellular PRL re-

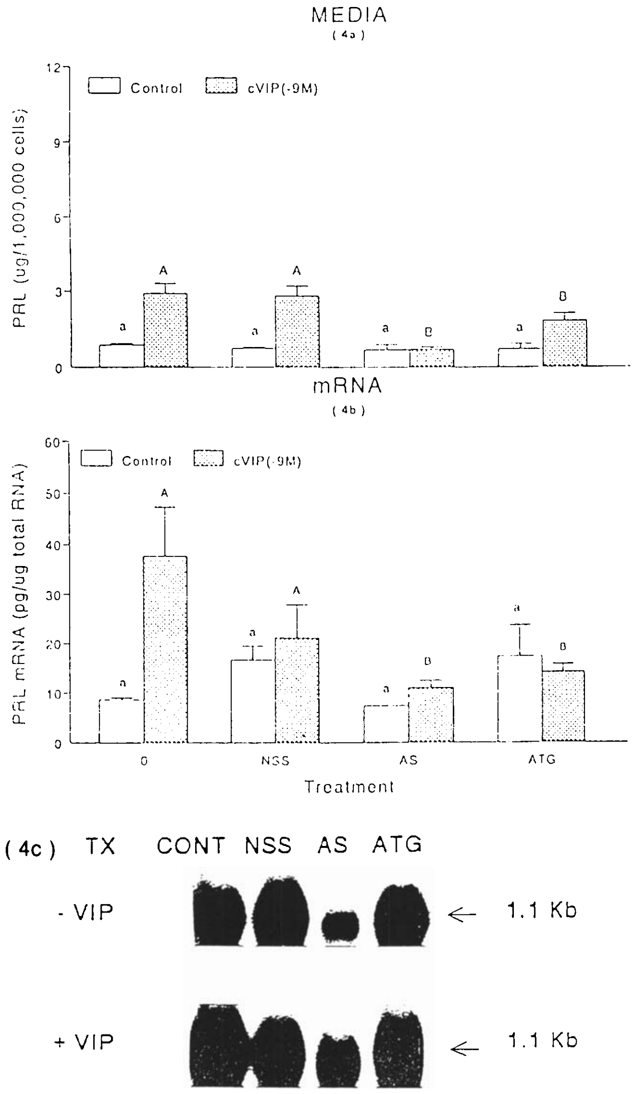


Figure 4. Stimulation of PRL secretion (a) and PRL mRNA levels (b) of cultured turkey anterior pituitary cells induced by 10^{-9} *M* chicken VIP. Data obtained in the presence or absence of 10^{-5} *M* VIP receptor antagonist (ATG; 4Cl-D-Phe^6,Leu^7-VIP), normal sheep serum (NSS; 1:5000), or VIP antiserum (AS; 1:500). Means + SEM are shown. Significant differences ($P < 0.05$) among groups within control and VIP-stimulated treatments are identified by different lowercase and capital letters, respectively. There were four replicate tubes/treatment and $n = 2$ experiments, with the exception of PRL mRNA were three replicates were used. Northern hybridization analysis of cellular PRL mRNA is shown in Panel c. Each lane represents total RNA from 90% of the cells cultured.

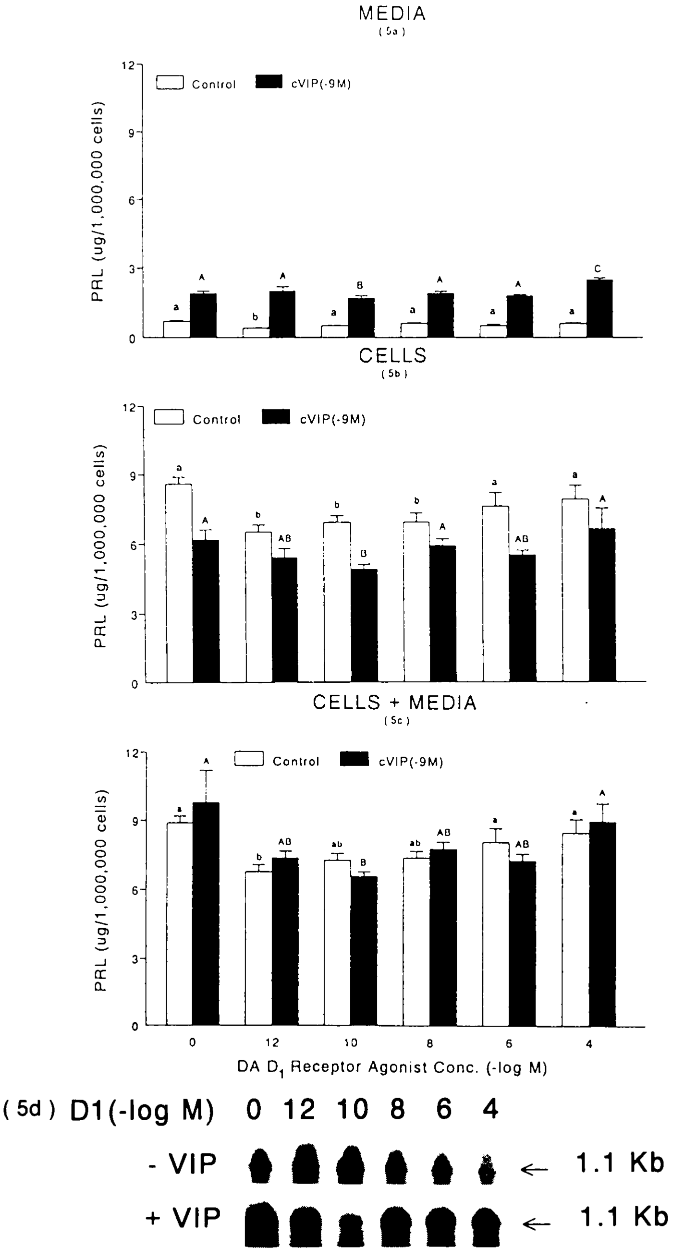


Figure 5. Effect of 10^{-9} *M* chicken VIP on PRL secretion (a), PRL cell content (b), and total PRL (c) of cultured turkey anterior pituitary cells in the presence or absence of different concentration of dopamine D_1 receptor agonist (SKF-38393). Means + SEM are shown. Significant differences ($P < 0.05$) among groups within control and VIP-stimulated treatments are identified by different lowercase and capital letters, respectively. There were four replicate tubes/treatment and $n = 2$ experiments, with the exception of PRL mRNA where three replicates were used. Northern hybridization analysis of cellular PRL mRNA is shown in Panel d. Each lane represents total RNA from 90% of the cells cultured.

sulting from VIP stimulation was blocked by the D_2 agonist (Fig. 7b). The marked increase in PRL mRNA levels induced by VIP was abolished by the D_2 agonist ($P < 0.002$; Fig. 7d).

Discussion

In these studies, we have examined the effects of VIP and of DA D_1 and D_2 receptor agonists in modulating the levels of PRL mRNA in turkey primary pituitary cells in culture. Prior studies have clearly demonstrated that VIP functions as a potent PRL-releasing hormone in turkeys and chickens, while DA exerts a relatively minor and variable effect on PRL secretion. We demonstrate here that VIP also stimulates steady-state PRL mRNA levels in cultured cells (presumably

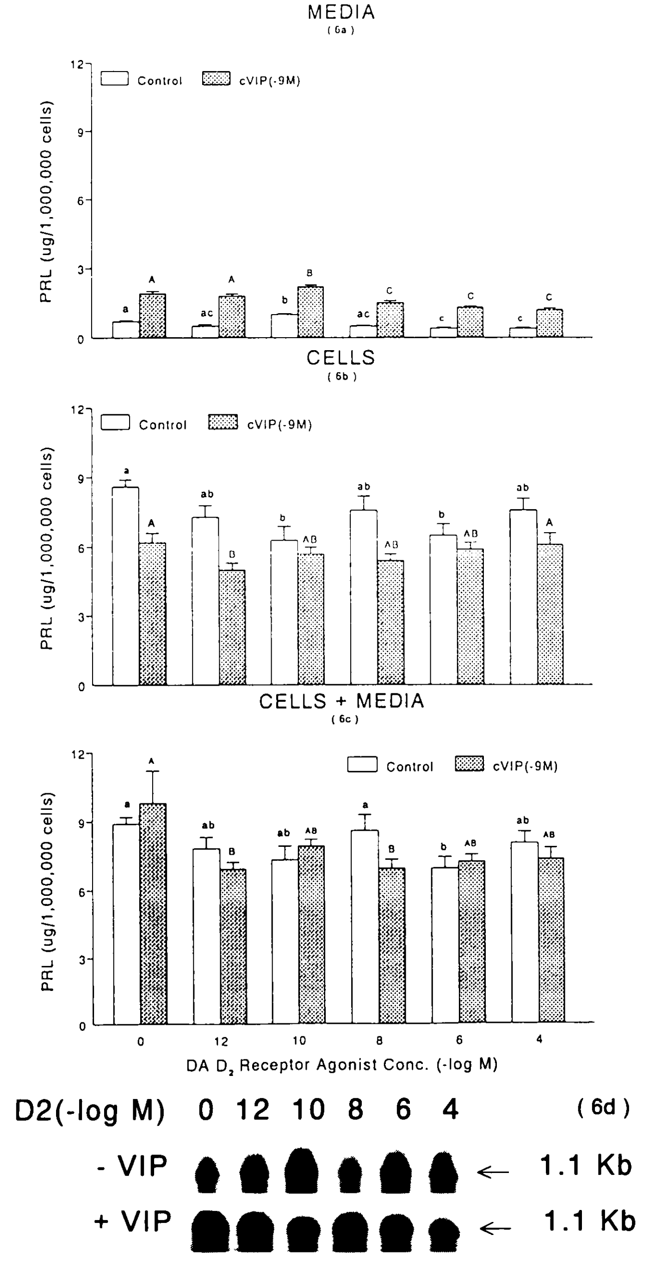


Figure 6. Effect of 10^{-9} *M* chicken VIP on PRL secretion (a), PRL cell count (b), and total PRL (c) of cultured turkey anterior pituitary cells in the presence or absence of different concentration of dopamine D_2 receptor agonist (quinpirole-HCl). Means + SEM are shown. Significant differences ($P < 0.05$) among groups within control and VIP-stimulated treatments are identified by different lowercase and capital letters, respectively. There were four replicate tubes/treatment and $n = 2$ experiments, with the exception of PRL mRNA where three replicates were used. Northern hybridization analysis of cellular PRL mRNA is shown in Panel d. Each lane represents total RNA from 90% of the cells cultured.

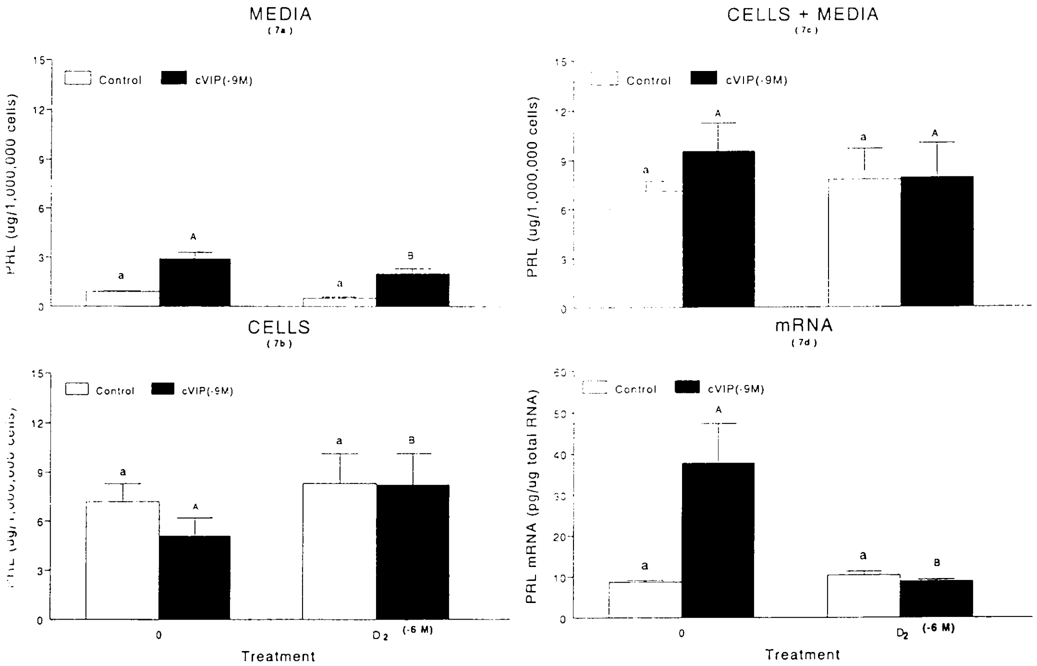


Figure 7. Effect of 10^{-9} *M* chicken VIP on PRL secretion (a), PRL cell content (b), total PRL (c), and PRL mRNA levels (d) of cultured turkey anterior pituitary cells in the presence or absence of 10^{-6} *M* dopamine D_2 receptor agonist (quinpirole-HCl). Means + SEM are shown. Significant differences ($P < 0.05$) among groups within control and VIP-stimulated treatments are identified by different lowercase and capital letters, respectively. There were four replicate tubes/treatment and $n = 2$ experiments, with the exception of PRL mRNA where three replicates were used.

through either increased mRNA synthesis or decreased degradation), and that the D_2 receptor may function to block the VIP-stimulated increase in PRL mRNA levels. We further show that this action of VIP on PRL mRNA is likely mediated by specific VIP receptors in the pituitary. The presence of high-affinity VIP receptors on turkey anterior pituitary cells was reported by Rozenboim and El Halawani (53). Antiserum to VIP blocked both the secretory response to VIP and the VIP-stimulated increase in PRL mRNA. Interestingly, VIP antiserum also appeared to reduce PRL mRNA levels in unstimulated cells, suggesting that endogenous VIP produced in the cultures may provide a stimulus to PRL synthesis. This suggestion is supported by our recent study (54) demonstrating the presence of turkey preproVIP in the pituitary. Mammalian studies have shown that VIP is synthesized in the anterior pituitary (55) that VIP mRNA levels in the pituitary are rapidly induced (56), and that treatment of rat pituitary cells with VIP antiserum suppresses PRL secretion, presumably through an autocrine mechanism (57).

Pituitary cells responded consistently to VIP, regardless of the number of cells used or the length of the incubation, confirming the reliability of the long-term cell culture system used in this study. Indeed, relatively short-term culture of pituitary cells appeared disadvantageous for studying the changes in PRL mRNA responses since PRL mRNA steady-state levels were very low as determined by Northern analysis during the first 36 hr following cell dispersal. Thereafter, the level of PRL mRNA and its response to VIP challenge increased dramatically, suggesting that the process of cell dispersion may downregulate PRL mRNA, and that lactotrophs may require a certain period of recovery before a response to VIP stimulation can be detected by this technique. Slot-blot analysis provided more sensitive detection of PRL mRNA than did Northern analysis, but results (Fig. 3d) also indicated that cells incubated less than 36 after dispersal were able to respond to VIP by secreting PRL although PRL mRNA levels did not always increase concomitantly.

Our observations on the time course of PRL response to continuous VIP treatment (Fig. 3) suggest that turkey pituitary cells do not readily become refractory to VIP stimulation. Vasoactive intestinal peptide stimulated PRL secretion within 3 hr of administration, and secretion increased steadily for 48 hr. This PRL release was associated with an initial decline (up to 12 hr) in cellular PRL content. However, concomitant with the increase in PRL mRNA levels, cellular PRL stabilized after 12 hr of exposure to VIP and increased between 48 and 72 hr. Total PRL (media + cells) increased steadily in VIP-stimulated cultures throughout the 72 hr of VIP exposure. After prolonged VIP stimulation, PRL mRNA abundance declined to the pre-stimulation level, perhaps due to being refractory to further stimulation, feedback of accumulating media PRL, or depletion of VIP in the media. Earlier findings in the rat have shown that a decrease in pituitary PRL content accompanies enhanced PRL secretion, and that the subsequent increase in PRL content is preceded by a transient increase in PRL mRNA levels (58).

Studies demonstrating inhibitory and stimulatory effects of DA and its agonists both *in vitro* and *in vivo* have been reported in mammals (24, 33). The inhibitory effects of DA are mediated by the D_2 subtype receptors (59), while a unique D_2 receptor may stimulate PRL release (34). Recent evidence suggests that this dual regulatory effect through the D_2 receptor also functions *in vivo* (33). Evidence to date for a role for DA in regulating PRL in the bird is controversial. Since PRL secretion is predominantly under stimulatory control, DA is not the potent regulator of PRL levels that it is in mammals and the roles of DA receptor subtypes are unexplored in birds. We report that the effect of the DA D_1 receptor agonist, SKF38393, on VIP-stimulated PRL secretion was variable and inconclusive. However, prior incubation of pituitary cells with the DA D_2 receptor agonist, quinpirole, diminished VIP-stimulated PRL secretion and PRL mRNA abundance at doses ranging from 10^{-8} to 10^{-4} *M*. At 10^{-10} *M*, quinpirole stimulated basal and VIP-induced PRL secretion. Although the exact mechanisms by which DA and its agonists affect PRL secretion in birds remain unknown, the present study provides evidence that DA D_2 receptor agonists are capable of regulating PRL release and PRL mRNA levels *in vitro*. Our demonstration that 10^{-6} *M* quinpirole abolished the 4-fold VIP-induced stimulation of PRL mRNA while having a less pronounced effect on PRL secretion suggests that the inhibitory effect of the agonist may occur through multiple mechanisms. Dopamine may also affect hypothalamic VIP, the putative PRL-releasing factor in the turkey (15, 17, 23). However, the demonstration of a direct effect of DA on the VIPergic system in birds is lacking. Treatment of the rat anterior pituitary with DA agonists has been shown to decrease VIP peptide and mRNA content (60), indicating that DA may have a negative effect on VIP synthesis and release. This suggests a potential mechanism for hypothalamic action *via* dopaminergic pathways.

In summary, utilizing an *in vitro* procedure with cultured pituitary cells, we studied the interaction between VIP and DA receptor agonists on PRL secretion, PRL mRNA content, and PRL synthesis. This study provides evidence that VIP, in addition to acting as a PRL-releasing factor, also play a major role in the regulation of PRL gene expression. Dopamine D_2 receptor agonist reduced the VIP-stimulated PRL release as well as PRL mRNA, but generally had no effect on basal PRL levels. Therefore, DA appears to exert its influence on the steady-state levels of PRL mRNA and the release of PRL primarily at the interaction of the releasing factor with the pituitary cells. The intracellular processing scheme from PRL synthesis to secretion is a complex process that may be regulated at multiple steps. The VIP-DA-induced changes in the steady-state level of PRL mRNA could be the result of alterations in gene transcription, nuclear mRNA processing, or cytoplasmic mRNA stability.

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